Clin. exp. Immunol. (1986) **66**, 481–489

Studies on the specificity of antibodies to ovalbumin in normal human serum: technical considerations in the use of ELISA methods

P. J. KILSHAW, FIONA J. McEWAN, K. C. BAKER & A. J. CANT *Department of Food Quality and Human Nutrition, AFRC Institute of Food Research, Reading Laboratory*

(Accepted for publication 26 June 1986)

SUMMARY

As part of a study designed to reveal information about molecular features of allergenic food proteins after absorption from the gut the specificity of antibodies in normal human serum to hen's egg ovalbumin was investigated using ELISA techniques. Preliminary investigations with monoclonal antibodies and hyperimmune rabbit antiserum specific for ovalbumin in its native and denatured form established that the molecule underwent an extensive conformational change on adsorption to polyvinyl chloride microtitre plates. The native conformation could be retained by using antibodies to couple the protein to the surface. Serum from 90% of healthy adult human donors contained IgG antibodies to ovalbumin. In nearly all cases the antibodies were specific predominantly for the native molecule and could not be absorbed with denatured ovalbumin or peptides prepared from it by cleavage with cyanogen bromide or trypsin. Antibodies to denatured ovalbumin were detected in most sera but at very low levels and were preferentially absorbed by the homologous antigen; peptides and native ovalbumin showing variable absorptive activity. Thus, although ovalbumin is ingested largely in a denatured form, the serum antibody response is stimulated mainly by topographic epitopes of the native molecule.

Keywords ovalbumin man specificity ELISA

INTRODUCTION

The absorption of food proteins in trace quantities after a meal is a normal physiological event. Absorbed proteins can be detected in the blood by a variety of sensitive immunoassays and can evoke antibody production or oral tolerance (Newby & Stokes, 1984) and, in certain sensitized subjects, elicit symptoms of food allergy both in the gut and in distant target organs (Lessof, 1984). Unfortunately very little is known about the molecular structure of such proteins that have endured food preparation, digestive processes and passage through the intestinal mucosa. Food-derived peptides entering the blood may exert important immunological or pharmacological activities but their detection and analysis have posed formidable technical difficulties (Gardner, 1984).

One approach that may reveal information about molecular features of absorbed food proteins would be to investigate the fine specificity of antibodies produced against them. This could help in understanding why certain foods are particularly allergenic. As a first step towards this goal we have studied the specificity of IgG antibodies to ovalbumin in sera sampled from a group of normal women. Ovalbumin is a particularly suitable choice as it is a very important food allergen in man (Langland, 1982; Hoffman, 1983; Cant, Marsden & Kilshaw, 1985) and has been widely used in

Correspondence: Dr P. J. Kilshaw, Department of Food Quality and Human Nutrition, AFRC Institute of Food Research, Shinfield, Reading, Berks RG2 9AT.

experimental animal models of hypersensitivity and oral tolerance (Newby & Stokes, 1984). In this paper we report the reactivity of such antibodies with the antigen in its native and denatured conformation and with peptides prepared from it by treatment with cyanogen bromide or trypsin.

In common with several other groups (Scott, Fausa & Brandtzaeg, 1984; Layton & Stanworth, 1984; Finn *et al.*, 1985) we have detected the antibodies to this protein with an enzyme-linked immunosorbent assay (ELISA). Because of recent reports that certain proteins undergo major conformational changes on adsorption to ELISA plates (Friguet, Djavadi-Ohaniance & Goldberg, 1984; Vaidya, Dietzler & Ladenson, 1985) it has been necessary first to investigate ovalbumin in this regard. This has been approached using a series of monoclonal antibodies prepared against the protein in its native and denatured state and with a conventional rabbit antiserum.

MATERIALS AND METHODS

Ovalbumin. Ovalbumin (OA, Grade VII) was obtained from Sigma, London. It contained a small proportion of denatured ovalbumin which was removed by hydrophobic interaction chromatography immediately before use. The protein (1 mg) was dissolved in 0·2 ml phosphate buffered saline (PBS) at twice normal strength (0·24 M NaCl, 0·06 M phosphate, pH 7·3) and applied to a column of phenyl sepharose (Pharmacia) prepared in a Pasteur pipette. Native OA was eluted from the column and collected in a glass vial, taking great care to avoid surface bubbles; denatured OA remained bound to the column.

Intentional denaturation was achieved by adding urea (8 M) to 300 mg OA in 20 ml 0·1 M Tris-HCl buffer, pH 8·2. Disulphide bonds were reduced by treatment with 2-mercaptoethanol (0·2 M) overnight at room temperature under nitrogen gas and then alkylated by addition of sodium iodoacetate (0·3 M), maintaining the pH at 8·0 for 2 h. The denatured protein was dialysed into PBS and the slightly opalescent solution was stored frozen at −20°C and centrifuged at 10,000 *g* for 10 min at room temperature immediately before use.

Monoclonal antibodies. Denatured or native OA (50 μg in Freund's complete adjuvant) was injected intraperitoneally into Balb/c mice and the injections repeated 3 weeks later. After a further 4 weeks the animals were boosted intravenously with a similar dose in saline and their spleen cells fused with the plasmacytoma P3X63Ag8,653 3 or 4 days later. Fusion and selection techniques were essentially as described by Galfrè & Milstein (1981) and cloning was performed by limiting dilution in 96-well culture plates (Nunc) using Balb/c peritoneal exudate macrophage feeder layers. Culture supernatants were screened by ELISA (see below). Selected hybridomas (10^6 to 5×10^6 cells) were injected i.p. into Balb/c mice and ascites fluid was harvested approximately two weeks later. IgG was isolated using staphylococcal protein A-separose and the Bio-Rad buffer system (Bio-Rad, Watford, UK).

Six hybridoma lines were prepared against native OA (NOA-1 to NOA-6) and five against the denatured molecule (DOA-1 to DOA-5). Epitope specificity was determined using a competitive inhibition radioimmunoassay (Stahli *et al.*, 1983). Four monoclonal antibodies were used in this study, DOA-1 and DOA-5, which bound only to the denatured protein and defined two separate epitopes, and NOA-2 and NOA-5, which were specific for native OA and, again, defined separate epitopes.

Rabbit antiserum to ovalbumin. A 1 mg dose of OA (Sigma, Grade V) in Freund's complete adjuvant was injected intramuscularly into New Zealand White rabbits. Injections were repeated 5 weeks later and the animals bled after a further 2 weeks. The antiserum was absorbed with ovomucoid (111–0, Sigma) coupled to Sepharose 4B and the antibodies affinity purified by elution at pH 2·2 from a mixture of native and denatured OA coupled to sepharose 4B.

Enzyme-linked immunosorbent assay (ELISA). Flat bottom polyvinyl chloride (PVC) microtitre plates (Dynatec) were coated overnight at 4°C with OA or rabbit IgG in PBS. The alkaline bicarbonate buffer usually employed for coating (Voller, Bidwell & Bartlett, 1976) was avoided because of the possibility of denaturing OA by high pH (Klausner *et al.*, 1983) and the use of PBS instead did not diminish protein adsorption. To detect antibodies to native OA, plates were coated first with affinity purified rabbit IgG specific for OA (1 μg/ml) or with monoclonal antibody (1 μg/ml

and then purified native ovalbumin (10 μg/ml) was added. In the assay for antibodies to denatured OA the antigen was coupled in this way also or, alternatively, coated directly to the plate. The quantity of OA adsorbed was predetermined using radioiodinated preparations (specific activity 0·5 mCi/mg) prepared with Bolton Hunter reagent. Duplicate samples of mouse, rabbit or human antibodies were applied to the coated plates (2 h at room temperature) and, finally enzyme-labelled anti-immunoglobulin was added for a similar period. PBS with 0·05% Tween 20 was used for washing plates and diluting reagents and, in assays involving a primary coating layer of rabbit immunoglobulin, contained in addition normal rabbit serum (1%). The following anti-immunoglobulin conjugates were used: horseradish peroxidase-labelled rabbit anti-mouse IgG (Dakopatts), alkaline phosphatase-labelled goat anti-rabbit IgG (Miles) and alkaline phosphatase-labelled goat anti-human IgG, γ-chain specific (Sigma). Substrates for peroxidase and phosphatase were, respectively, *o*-phenylenediamine (0·4 mg/ml in citrate/phosphate buffer, pH 5·0) and *p*-nitrophenylphosphate (1 mg/ml in diethanolamine buffer, pH 9·8).

Background values in the ELISA were determined using appropriate normal sera; for human antibody assays, a serum was chosen that gave very low assay values that could be reduced very little further by exhaustive absorption with OA.

Assays of human serum were standardized by including on each assay plate a series of wells containing radioiodinated normal human IgG (serial dilutions from 1 μg/ml; specific activity 0·6 μCi/μg). Results are expressed as ELISA units, defined as the dilution of serum giving an o.d. equivalent to 8ng IgG bound to an assay well. Because the efficiency of detection of antigen-bound and PVC-bound IgG may differ (Dierks, Butler & Richerson, 1986) our results are not expressed as absolute amounts of antibody per ml of serum.

Inhibition assays were performed by preincubating human sera (1:10), rabbit antibody (1 μg/ml) or monoclonal ascites fluid (1:5000) overnight at 4°C with inhibitors (OA or peptides) and the remaining antibody activity was then measured by ELISA.

Human sera. Thirty-eight lactating women abstained from egg for 12 h and then a blood sample was taken from each and the serum harvested and stored at −20°C.

Proteolysis by trypsin or cyanogen bromide. Denatured OA (6 mg/ml in 0·05 M Tris-HCl, pH 7·4) was digested for 15 min at 37°C with trypsin (X11-S, Sigma) at 1% (w/v) and the enzyme was then removed by addition of excess soyabean trypsin inhibitor coupled to Sepharose 4B. Cyanogen bromide cleavage was accomplished by incubating native OA (43 mg/ml in 70% formic acid) with cyanogen bromide (100-fold molar excess over methionine) for 24 h at room temperature.

Analysis of the products by SDS polyacrylamide gel electrophoresis suggested that cyanogen bromide had cleaved the molecule to give the expected peptides (Nisbet *et al.*, 1981), i.e. those of 132, 85, 32, 27 and 24 residues and additional small fragments. Digestion of denatured ovalbumin yielded a major fraction (mol. wt approximately 15000 daltons) and a range of smaller peptides.

RESULTS

Specificity of monoclonal antibodies for native and denatured OA. Monoclonal antibodies to native OA (NOA-2 and NOA-5) and denatured OA (DOA-1 and DOA-5) showed almost complete specificity for their homologous antigens when tested by ELISA (Fig. 1) and by ELISA inhibition (Fig. 2).

Conformational changes in native OA on adsorption to polyvinyl chloride microtitre plates. An assay plate was coated with denatured or native OA in a range of concentrations and then tested with monoclonal antibodies to the two forms of the protein. Figure 3 shows that monoclonal antibodies to denatured OA (DOA-1 and DOA-5) bound equally well to a coating of native or urea-denatured protein. In contrast, monoclonal antibodies to native ovalbumin (NOA-2 and NOA-5) gave negative results with the native molecule except at the very highest coating levels (25–100 μg/ml) and also failed to bind to denatured OA.

The apparent conformational change on adsorption of OA to PVC was observed also in an ELISA inhibition test using rabbit antiserum. Native or urea-denatured OA was coated directly onto an assay plate and then tested with rabbit antiserum that had been pre-absorbed with either

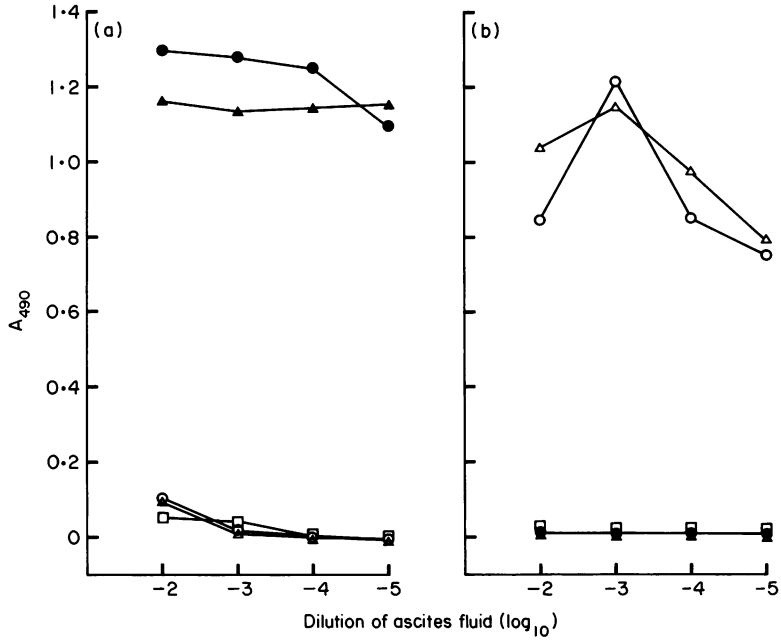

Fig. 1. Specificity of monoclonal antibodies for native and denatured OA tested by ELISA. (a) Native OA was coupled to an ELISA plate with rabbit antibody; (b) urea-denatured OA was adsorbed directly to the plate. The following monoclonal antibodies were then tested for binding: NOA-2 (▲), NOA-5 (●) DOA-1 (△) and DOA-5 (○). Normal mouse serum (□) was used as a control.

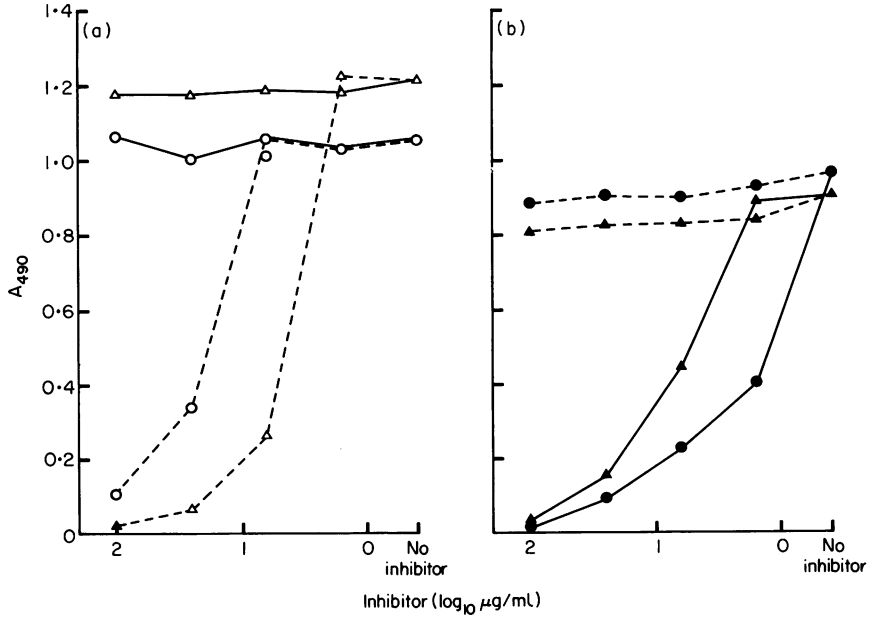

Fig. 2. Specificity of monoclonal antibodies determined by an ELISA inhibition test. Monoclonal antibodies NOA-2 (▲), NOA-5 (●), DOA-1 (△) and DOA-5 (○) in ascites fluid (1:5000) were absorbed overnight with native (—) or denatured (- - -) OA and then tested by ELISA against their homologous antigen. (a) Denatured OA; (b) native OA.

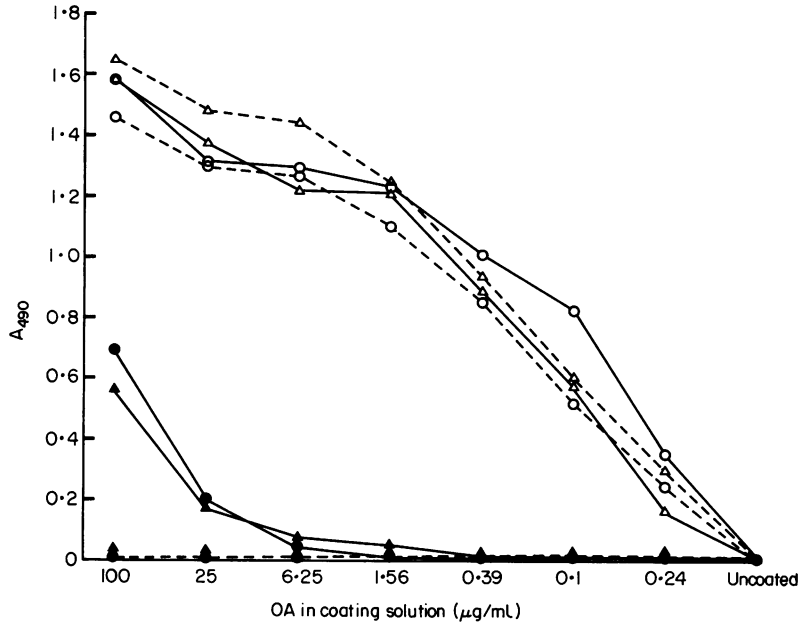

Fig. 3. Denaturation of OA on adsorption to an ELISA plate assessed with monoclonal antibodies. A PVC plate was coated directly with native (—) or denatured (- - -) OA in a range of concentrations and then tested with monoclonal antibodies NOA-2 (▲) and NOA-5 (●) or DOA-1 (△) and DOA-5 (○), specific for the native or denatured form respectively (ascites fluid 1:1000).

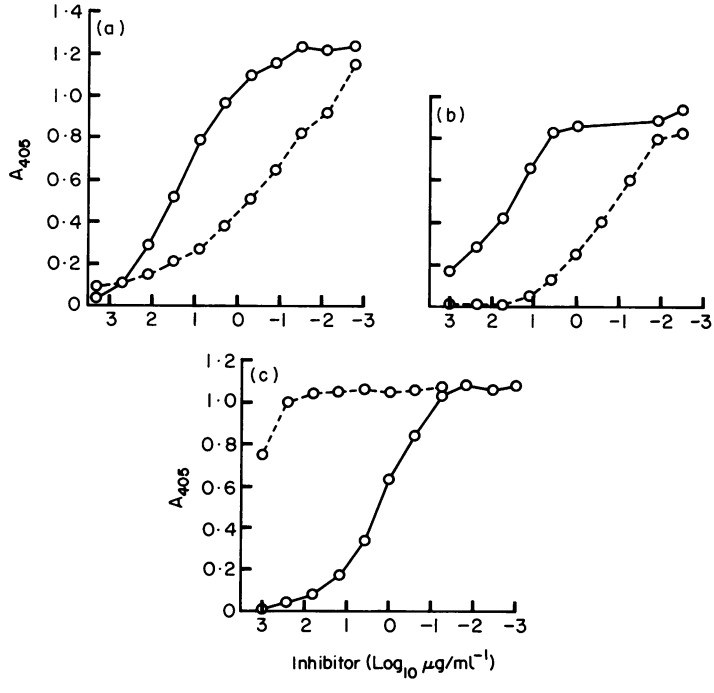

Fig. 4. Denaturation of OA on adsorption to an ELISA plate assessed with rabbit antiserum. Affinity-purified rabbit antibodies to OA (1 μg/ml) were absorbed overnight with native (—) or denatured (- - -) OA and then tested against (a) native OA adsorbed directly to an ELISA plate, (b) urea-denatured ovalbumin coated similarly and (c) native OA attached to the plate via the monoclonal antibody NOA-2.

Table 1. Assay of human sera for antibodies to native and denatured OA

	Antibody (ELISA units*) to:	
Serum donor	Native OA	Denatured OA
Ba	105	< 10
Un	100	< 10
Du	16	17
Be	95	< 10
Se	174	< 10
Gi	162	< 10
Hi	120	24
Yo	53	11
Co	182	< 10
Sw	110	< 10
Wi	120	< 10
Ha	417	< 10
Ko	55	< 10
Al	22	< 10
Jo	83	26
Ke	151	< 10
Ha	158	< 10
Fa	36	< 10
Du	63	< 10
Sa	48	< 10
Be	398	< 10
Ri	14	< 10
Cl	28	< 10

* ELISA units defined as the dilution of serum required to achieve binding of 8 ng IgG antibody per assay well.

preparation. The two coatings gave closely similar results, urea-denatured OA being by far the more effective inhibitor (Fig. 4a, b). In contrast, when native OA was attached to the plate by monoclonal antibody (NOA-2), native OA inhibited strongly and the urea-denatured preparation was almost ineffective (Fig. 4c).

Antibodies in human sera to native and denatured OA. Twenty-five sera were tested by ELISA for antibodies to OA in its native and denatured form. Assay conditions were identical in that both forms of the protein were coupled to ELISA plates with rabbit antibodies and were present at a level of 10 ng per assay well. Twenty-three sera showed antibody activity to the native molecule but little or none to the denatured form (Table 1). Direct attachment of denatured OA to the assay plates at the same level gave similar results but when the coating was increased to 300 ng per assay well, sensitivity was enhanced and most sera showed sufficient antibody binding to permit inhibition studies.

Absorption of sera with native and denatured OA and with peptides. Twenty-one sera containing antibodies reactive with both forms of OA were retested after absorption with native or denatured protein or peptides derived from it by treatment with cyanogen bromide or trypsin. Results with the maximum concentration of inhibitor used (50 μg/ml) are shown (Fig. 5). Antibodies to native OA were absorbed completely by the native form whereas peptides and denatured OA were ineffective. Antibodies to urea-denatured OA were absorbed most effectively by the homologous antigen but peptides and the native protein showed a range of inhibitory activity.

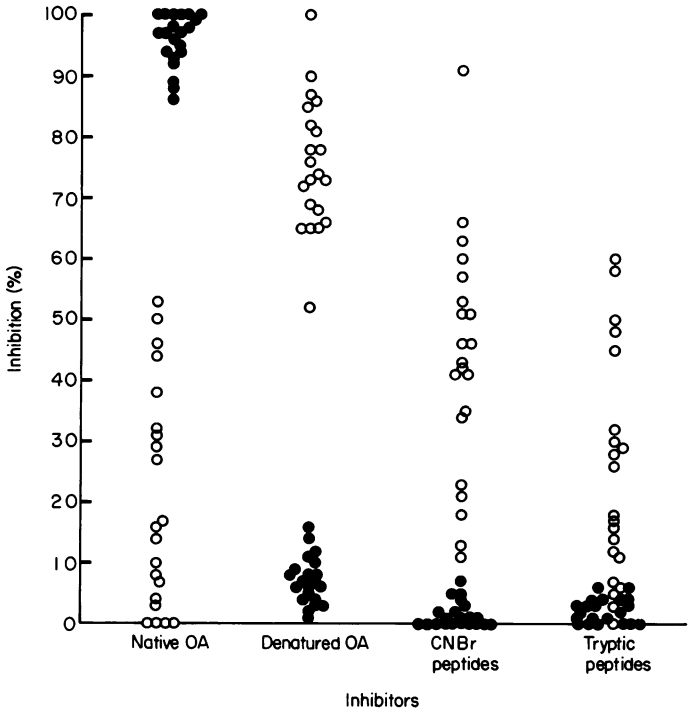

Fig. 5. ELISA inhibition test on human sera. Inhibitory activity of native and denatured OA and peptides (all at 50 μg/ml) in assays for antibodies to the native (●) or denatured (○) molecule.

DISCUSSION

Since their inception ELISA techniques have enjoyed great popularity because of their speed, simplicity and cheapness. Recently, however, experiments with monoclonal antibodies in several laboratories have identified conformational changes in certain proteins adsorbed to ELISA plates (Friguet *et al.*, 1984; Vaidya *et al.*, 1985). This has prompted recommendations by these workers that antibodies which are being developed to react with antigens in solution should not be screened by techniques that immobilize the antigen directly to a plastic surface. Our experiments with OA confirm that major conformational changes can take place and, indeed, our monoclonal antibodies to four epitopes and a conventional rabbit antiserum failed to distinguish between urea-denatured OA and native OA after adsorption to PVC. At very high coating levels, epitopes of the native molecule were detected. A plausible explanation is that at such concentrations binding sites on the plate are saturated and that additional binding involves layering and protein-protein interactions (Cantarero, Butler & Osborne, 1980) that do not denature the molecule.

On dialysis following treatment with 8 M urea OA forms an alternative structure with antigenic properties quite distinct from those of the native protein and markedly increased hydrophobicity (Klausner *et al.*, 1983). This change occurs regardless of the integrity of disulphide bonds and suggests that the native conformation has a higher free energy and is formed by sequential folding during translation. The present experiments suggest that hydrophobic bonding to PVC evokes a similar molecular transformation. We have observed this change in OA also to occur on adsorption to polystyrene, during Western blotting onto nitrocellulose, on heat treatment at 75°C and, to a lesser degree, on coupling the molecule to CNBr-activated cellulose paper in the preparation of RAST discs. The practical problem of using an ELISA technique to detect antibodies to OA in its native form was solved by attaching the antigen to the plates with polyclonal rabbit antibodies. Kemeny *et al.* (1985) have observed that this method of antigen attachment greatly amplifies the sensitivity of an ELISA for the detection of IgE antibodies, an effect that may well be due to

avoidance of antigen denaturation. It is possible that polymerization of native ovalbumin with glutaraldehyde before coating to an ELISA plate may also prevent denaturation for it has been reported that this strategy is necessary when using an ELISA plaque technique to detect cells producing antibodies to native ovalbumin (Holt *et al.*, 1984; Sedgwick & Holt, 1986).

Ovalbumin is known to be a particularly important food allergen and the main objective of this study was to gain information about the specificity of antibodies to it. Egg in the diet is likely to be the principal, if not exclusive, source of antigenic exposure to ovalbumin and our observation that nearly all the serum samples contained IgG antibodies to it is consistent with the results of others (Husby *et al.*, 1985).

The choice of lactating women as experimental subjects followed logically from earlier work in this laboratory on the absorption of dietary proteins during lactation and their transfer into breast milk (Kilshaw & Cant, 1984). We have not yet investigated antibody specificity in men and non-lactating women but have no reason to suspect that it may differ.

Standardization of our ELISA systems allowed a precise comparison to be made between antibodies to the two forms of ovalbumin. This showed that the response to the native molecule was predominant in quantity or affinity. The ELISA inhibition studies revealed that these antibodies were specific mainly for topographic epitopes that are not represented on peptides or the denatured molecule. Native ovalbumin, in contrast to the denatured form, is rather resistant to tryptic proteolysis (Yokota, Matsushima & Inada, 1982), an observation repeatedly confirmed in our own laboratory. Undoubtedly ovalbumin in the diet is substantially denatured, thus the observed antibody specificity may be a consequence of absorption of a minor, relatively indigestible, fraction of the protein.

The present study does not consider the specificity of IgE antibodies to ovalbumin. Our results for IgG stand in sharp contrast to those of Elsayed *et al.* (1986) for IgE, in which denatured and native ovalbumin were equally effective in inhibiting a radioallergosorbent test. It is conceivable that this apparent difference in specificity between the two isotypes may reflect an important dichotomy in antigen handling and in the microenvironment of the antigen during absorption. However, it is notable that in the latter study the molecular conformation of ovalbumin on the RAST discs was not investigated so the comparison may be ill-founded.

Little is known about the fine specificity of antibodies to food allergens. Further work in this area should improve knowledge of antigen handling in the gut and assist the design of serological tests for food sensitivity. The molecular conformation of antigens immobilized on solid surfaces in antibody assays seldom receives attention. Our results with ovalbumin highlight afresh this important technical consideration.

The authors thank the Medical Research Council for supporting K.C.B.

REFERENCES

CANT, A., MARSDEN, R.A. & KILSHAW, P.J. (1985) Egg and cow's milk hypersensitivity in exclusively breast-fed infants with eczema, and detection of egg protein in breast milk. *Br. med. J.* **291,** 932.

CANTARERO, L.A., BUTLER, J.E. & OSBORNE, J.W. (1980) The adsorptive characteristics of proteins for polystyrene and their significance in solid phase immunoassays. *Anal. Biochem.* **105,** 375.

DIERKS, S.E., BUTLER, J.E. & RICHERSON, H.B. (1986) Altered recognition of surface-adsorbed compared to antigen-bound antibodies in the ELISA. *Molec. Immunol.* **23,** 403.

ELSAYED, S., HAMMER, A.S.E., KALVENES, M.B., FLORVAAG, E., APOLD, J. & VIK, H. (1986) Antigenic and allergenic determinants of ovalbumin. 1. Peptide mapping, cleavage at the methionyl peptide bonds and enzymic hydrolysis of native and carboxymethyl OA. *Int. Archs. Allergy appl. Immun.* **79,** 101.

FINN, R., HARVEY, M.M., JOHNSON, P.M., VERBOV, J.L. & BARNES, R.M.R. (1985) Serum IgG antibodies to gliadin and other dietary antigens in adults with atopic eczema. *Clin. exp. Dermatol.* **10,** 222.

FRIGUET, B., DJAVADI-OHANIANCE, L. & GOLDBERG, M.E. (1984) Some monoclonal antibodies raised with native protein bind preferentially to the denatured antigen. *Molec. Immun.* **21,** 673.

GALFRÈ, G. & MILSTEIN, C. (1981) Preparation of monoclonal antibodies: strategies and procedures. In: *Methods in Enzymology* Vol. 73. *Immunochemical Techniques (part C)* (ed. by J.J. Langone & H. Van Vunakis) p. 3. Academic Press, New York.

GARDNER, M.L.G. (1984) Intestinal assimilation of

intact peptides and proteins from the diet—a neglected field? *Biol. Rev.* **59,** 289.

HOFFMAN, D.R. (1983) Immunochemical identification of the allergens in egg white. *J. Allergy clin. Immunol.* **71,** 481.

HOLT, P.G., SEDGWICK, J.D., STEWART, G.A., O'LEARY, C. & KRSKA, K. (1984) ELISA plaque assay for the detection of antibody secreting cells: observations on the nature of the solid phase and on variations in plaque diameter. *J. immunol. Methods* **74,** 1.

HUSBY, S., OXELIUS, V.A., TEISNER, B., JENSENSIUS, J.C. & SVEHAG, S.E. (1985) Humoral immunity to dietary antigens in healthy adults. Occurrence, isotype and IgG subclass distribution of serum antibodies to protein antigens. *Int. Archs. Allergy appl. Immun.* **77,** 416.

KEMENY, D.M., URBANEK, R., SAMULE, D. & RICHARDS, R. (1985) Improved sensitivity and specificity of a sandwich ELISA for measurement of IgE antibodies. *J. immunol. Methods* **78,** 212.

KILSHAW, P.J. & CANT, A.J. (1984) The passage of maternal dietary proteins into human breast milk. *Int. Archs. Allergy appl. Immun.* **75,** 8.

KLAUSNER, R.D., KEMPF, C., WEINSTEIN, J.N., BLUMENTHAL, R. & VAN RENSWOUDE, J. (1983) The folding of ovalbumin. Renaturation in vitro versus biosynthesis in vivo. *Biochem. J.* **212,** 801.

LANGLAND, T.A. (1982) Clinical and immunological study of allergy to hens egg white. 11. Antigens in hens egg white studied by crossed immunoelectrophoresis (CIE). *Allergy* **37,** 323.

LESSOF, M.H. (1984) Food intolerance and food aversion. A joint report of the Royal College of Physicians and the British Nutrition Foundation. *J. Royal College of Physicians of London* **18,** 1.

LAYTON, G.T. & STANWORTH, D.R. (1984) The quantitation of IgG4 antibodies to three common food allergens by ELISA with monoclonal anti-IgG4. *J. immunol. Methods* **73,** 347.

NEWBY, T.J. & STOKES, C.R. (eds) (1984) *Local Immune Responses in the Gut.* CRC Press, Inc., Florida.

Nisbet, A.D., Saundry, R.H., Moir, A.J.G., Fothergill, L.A. & Fothergill, J.E. (1981) The complete amino acid sequence of hen ovalbumin. *Eur. J. Biochem.* **115,** 335.

SCOTT, H., FAUSA, O., EK, J. & BRANDTZAEG, P. (1984) Immune response patterns in coeliac disease. Serum antibodies to dietary antigens measured by an enzyme linked immunosorbent assay (ELISA). *Clin. exp. Immunol.* **57,** 25.

SEDGWICK, J.D. & HOLT, P.G. (1986) The ELISA-plaque assay for the detection and enumeration of antibody-secreting cells: an overview. *J. Immunol. Methods* **87,** 37.

STÄHLI, C., MIGGIANO, V., STOCKER, J., STAEHELIN, TH., HÄRING, P. & TAKACS, B. (1983) Distinction of epitopes by monoclonal antibodies. In: *Methods in Enzymology* Vol. 92. *Immunochemical Techniques (part E)* (ed. by J.J. Langone & H. Van Vunakis) p. 242. Academic Press, New York.

VAIDYA, H.C., DIETZLER, D.N. & LADENSON, J.H. (1985) Inadequacy of traditional ELISA for screening hybridoma supernatants for murine monoclonal antibodies. *Hybridoma* **4,** 271.

VOLLER, A., BIDWELL, D.E. & BARTLETT, A. (1976) Enzyme immunoassays in diagnostic medicine, theory and practice. *Bull. WHO* **53,** 55.

YOKOTA, Y., MATSUSHIMA, A. & INADA, Y. (1982) Isolation of peptides with immunoreactivity from ovalbumin by trypsin digestion. *FEBS Letters* **141,** 225.